

MOM_Load

1, add the whole folder of the code into the Matlab pathway;

2, run 'MOM_Load_11_3';

3, 'Selection regressor list' → 'Custom list' → select 'BigArenaOutdated.rlf';

4, click pushbutton 'Load' → select an eyetracker file (e.g., 'Rec1_-50um_GCaMP5G_5dpf_20170602_newedited.txt') → select a corresponding avi file (e.g. 'rec1_-50um_20170602_laser17_gain_560_mag2_Gcamp5g_5dpf_2fps_zeissobj_smallbulb_righteye_NtoT_fish1_rot_reg').

5, click 'Create Zscore map'

6, click 'Draw ROI'

7, type in the number '2' and press 'Enter' key when the 'Use suprathreshold Z picture?' shows up;

8, manually select one ROI on the 'figure 5' in the highlighted region (warm color in red or yellow);

9, type in a '1' (any positive integer in this version) and press the 'Enter' key when the 'Outline Another ROI? (1 = yes, 0 = no)' shows up and you want to select another ROI. Repeat step number '8';

9, type in the number '0' and press 'Enter' key when the 'Outline Another ROI? (1 = yes, 0 = no)' shows up and you want to finish. (The analysis ends here and the data have been saved in the figures and .mat files)

10, (the datasets need to be analyzed one by one)

Midbrain_localizer_V0_4

('CellViewer' is a sub-function of 'Midbrain_localizer'; the main code of 'Midbrain_localizer_V0_4' can only run with lower version of Matlab (Matlab 2010 and lower versions); Matlab version 2014 is also compatible to the code if you only use the 'Cellviewer' part)

1, add the whole folder of the code into the Matlab pathway and run the 'Midbrain_localizer_V0_4' main function;

2, click pushbutton 'Load zstack' and load a zstack file (e.g., the example file, 'zstack1_20170602_laser17-24_gain_600_mag2_Gcamp5g_5dpf_2fps_zeissobj_-90-70_fish1_rot.tif');

3, click pushbuttons 'rotation' and 'Show figure' in order; slide the 'vSlice' to the right side until the 'vSlice #' shows number 1;

4, optimize the new figure in the new window; e.g., the pixel intensity and image contrast are adjustable with 'low_in', 'high_in' and 'gamma' values;

5, draw the boundary between tectum and pretectum on the new figure from the lower left to the lower right. There are two optional methods: **(A)**, drawing the boundary freely with a curve (click 'Draw_Curve', start drawing by left click, then draw the boundary manually and end the plot by right click) and **(B)**, drawing the boundary with a closed polygon (the initial default drawing is shown on the figure in polygon format. The position of each dot can be moved manually by the user; click 'draw_poly' and start changing the position of the dots; finish by double click) (see **Figure 1 A-B**); the newly drawn boundary (a curve or a polygon) will be shown on next layer as a reference of the boundary for the user;

6, three new figures with different methods to smooth the curve from step 5 show up; choose the method matching the original data (in blue) and fitted data (in red) the best by checking the checkbox (Gauss3 is the default method); click pushbutton 'save' if the boundary has been successfully drawn. Otherwise, click the 'vSlice' to another layer then click it again back to the intended layer. Draw the boundary with either of the methods mentioned in step 5;

7, click pushbutton 'vSlice' to the next layer to activate the two buttons 'Draw_Curve' and 'draw_poly';

8, repeat steps 5 to 7 to draw the boundary slice by slice (if the boundaries from several slices are the same, users just need to draw and save the boundary for the left most slice and the others will be generated and saved automatically) ;

9, click pushbuttons 'show ori boundary' and 'Export boundaries' to export the original boundary;

10, click pushbuttons 'New Landmarks'; a new window of a zstack image shows up;

11, find the landmark of posterior commissure and draw a line (by clicking mouse twice separately) from the bottom to the top across the midline of the fish. The start and the end points should be located at the rostral tips of the pretectal neuropils in the image (**Figure 2A**);

12, enter number '1' if the line in step 11 has been correctly drawn. Otherwise, enter number '0' and repeat step 11;

13, find the same layer as in step 11 with the landmark of posterior commissure and draw a line along the midline of the fish (**Figure 2B**);

14, enter number '1' if the line in step 13 has been correctly drawn. Otherwise, enter number '0' and repeat step 13;

15, find the landmark of the joining point of the tectal and pretectal neuropils on the left side of the fish brain (roll the slider downwards) and draw a tilt line along the pretectal neuropil (**Figure 2C**);

16, enter number '1' if the line in step 15 has been correctly drawn. Otherwise, enter number '0' and repeat step 15;

17, find the landmark of the joining point of the tectal and pretectal neuropils on the right side of the fish brain (roll the slider upwards) and draw a tilt line along the pretectal neuropil (**Figure 2D**);

18, enter number '1' if the line in step 17 has been correctly drawn. Otherwise, enter number '0' and repeat step 17;

19, find the midline of the fish and draw a line from the left side to the right side of tectal neuropils, perpendicular to the midline (**Figure 2E**);

20, enter number '1' if the line in step 19 has been correctly drawn. Otherwise, enter number '0' and repeat step 19;

21, click pushbutton 'Thr_D_boundary' to save the tectum-pretectum boundary and 'gvars' values (these two datasets are quite useful if Matlab crashes in the following steps).

22, click pushbutton 'LoadSlice files' and choose an image time series in .tif format and load the corresponding 'RegionStats' file from the 'MOMLoad' analysis.

23, click pushbutton 'Show Alignment' and a new window with three figures appears; adjust 'xOffset', 'yOffset' and 'Slice#' which correspond to the adjustments in x, y and z dimensions of the zstack to match the first two figures with each other as shown in the last figure (**Figure 3**);

24, select all the cells in the Midbrain_localizer main GUI and click pushbuttons 'Show Selected ROIs' to check the location of the cells (**Figure 4**).

25, click pushbutton 'Export ROIs' to export the data;

26, repeat the step 22 to 25 with other datasets;

27, (optional) click the pushbutton 'PoolExportCells' and select the datasets, which you just exported, and save them in one file.

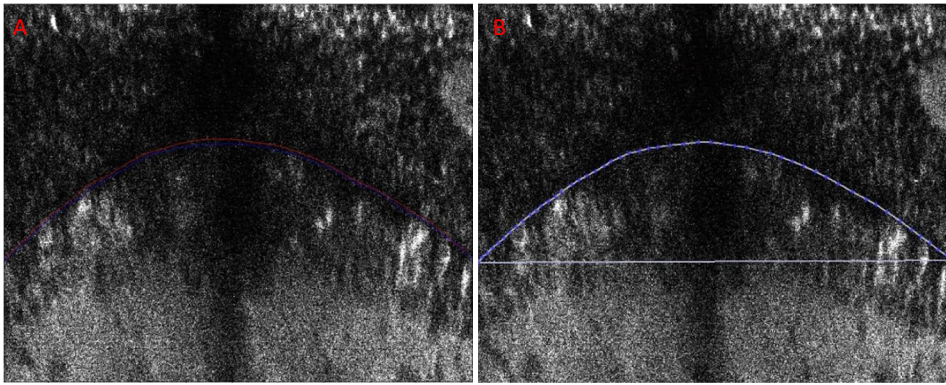


Figure 1. Tectum-pretectum boundaries drawn with 'Draw_Curve' (A) and 'draw_poly' (B); (A) the red and the blue curves are the newly (current layer) and previously (previous layer) drawn boundaries in two different layers. (B) a boundary drawn with a polygon.

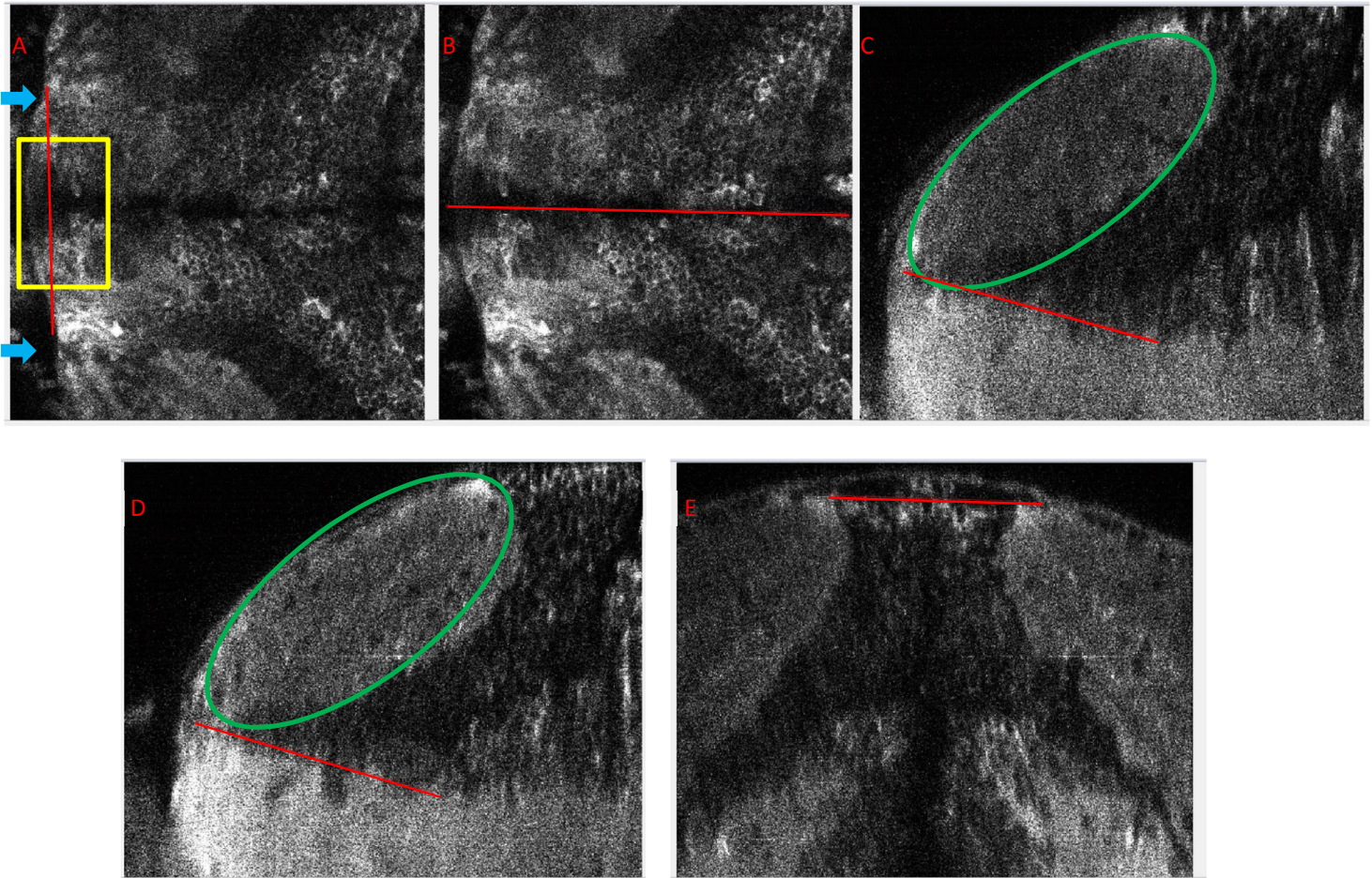


Figure 2. The manually drawn reference lines and the landmarks. (A) dorsal view, arrows indicate the starting points of the pretectal neuropils. Posterior commissure is enclosed in the yellow rectangle. The red line correspond to the line drawn in step 11; (B) dorsal view, the red line correspond to the line drawn in step 13; (C) (left hemisphere) and (D) (right hemisphere), side view, the neuropils are indicated with green ellipses and the red tilt lines are along the pretectal neuropils. (E) frontal view, the red line crosses the two hemispheres and is perpendicular to the midline (corresponding to the step 19).

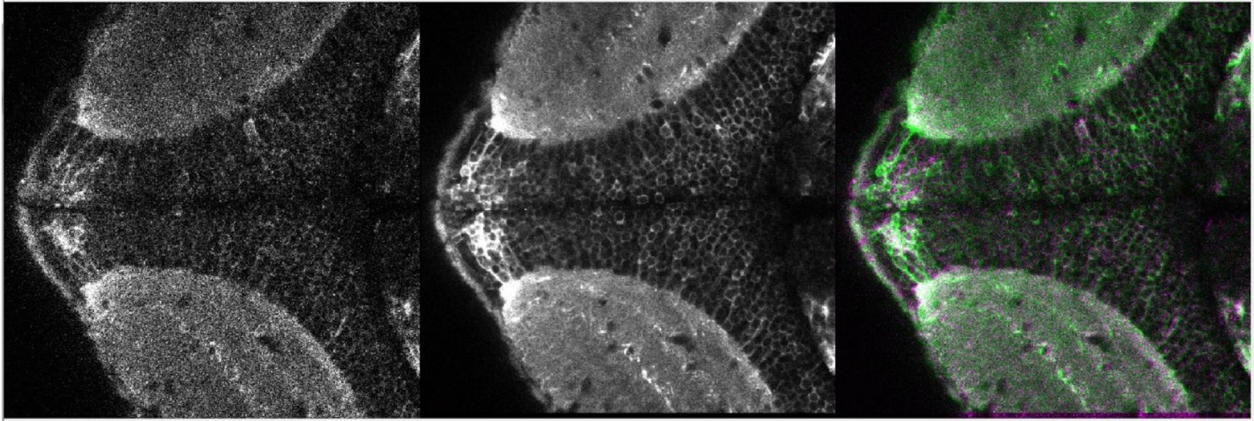


Figure 3. The three figures in step 23. Left panel, the visually chosen layer from the z-stack, which should be identical to the figure in middle panel; middle panel, mean of the first 30 frames from the calcium image time series; right panel, overlay of the figures from the left (green) and the middle (magenta) panels;

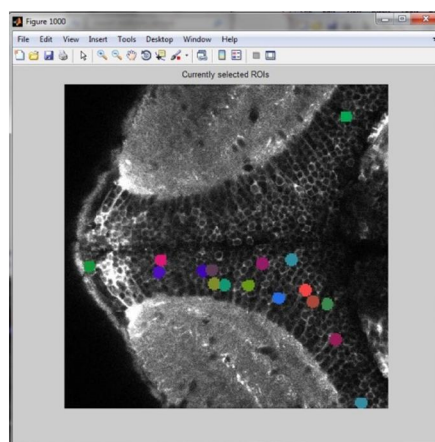


Figure 4. The cells in step 24 shown in 2D.

If Matlab crashes after step 21, please follow the steps below to continue the analysis.

- 1, add the whole folder of the code into the Matlab pathway and run the Midbrain_localizer_V0_4 main function;
- 2, click pushbutton 'Load zstack' and load a zstack file (e.g., the example file, 'zstack1_20170602_laser17-24_gain_600_mag2_Gcamp5g_5dpf_2fps_zeissobj_-90-70_fish1_rot.tif');
- 3, click pushbuttons 'rotation' and 'Show figure' in order; slide the 'vSlice' to the right side until the 'vSlice #' shows number 1;
- 4, click pushbutton 'Load_gvars', and select the 'gvars' file which was exported in step 21 above;
- 5, click pushbutton 'Load_Boundary', and select the full boundary file which was exported in step 21 above;
- 6, continue the analysis with the steps 22 to 27 of the protocol shown above.

CellViewer_V4_2

(this part can be run with Matlab 2010 and higher versions)

1, add the whole folder of the code into the Matlab pathway and run the Midbrain_localizer_V0_4

Main function;

1, 2, click pushbutton 'CellViewer';

3, click 'Load data file', select the data file 'Exportmat_Monocular_RFM.mat';

3, click pushbutton 'Load boundary file', select a boundary data file (e.g., 'zstack1_20170602_laser17-24_gain_600_mag2_Gcamp5g_5dpf_2fps_zeissobj_-90-70_fish1_rot_exported_boundary_of_brain_060817_160148.mat'; Any boundary file works here).

4, click pushbutton 'Highlight cells RFs';

5, change the settings as indicated in the following figure (**Figure 5 A**) and click 'Show cells (RF)'; Then the 3D plot of the tectal neurons colored according to the RF size appears. (the users can show 'all cells' or 'pretectum only' as well; in the red rectangles of Figure 5, 'RF center x' and 'RF center y' are also available to show the neurons according to locations of their RF centers (**Figure 5 B**))

6, click pushbutton 'export orthogonal view RF';

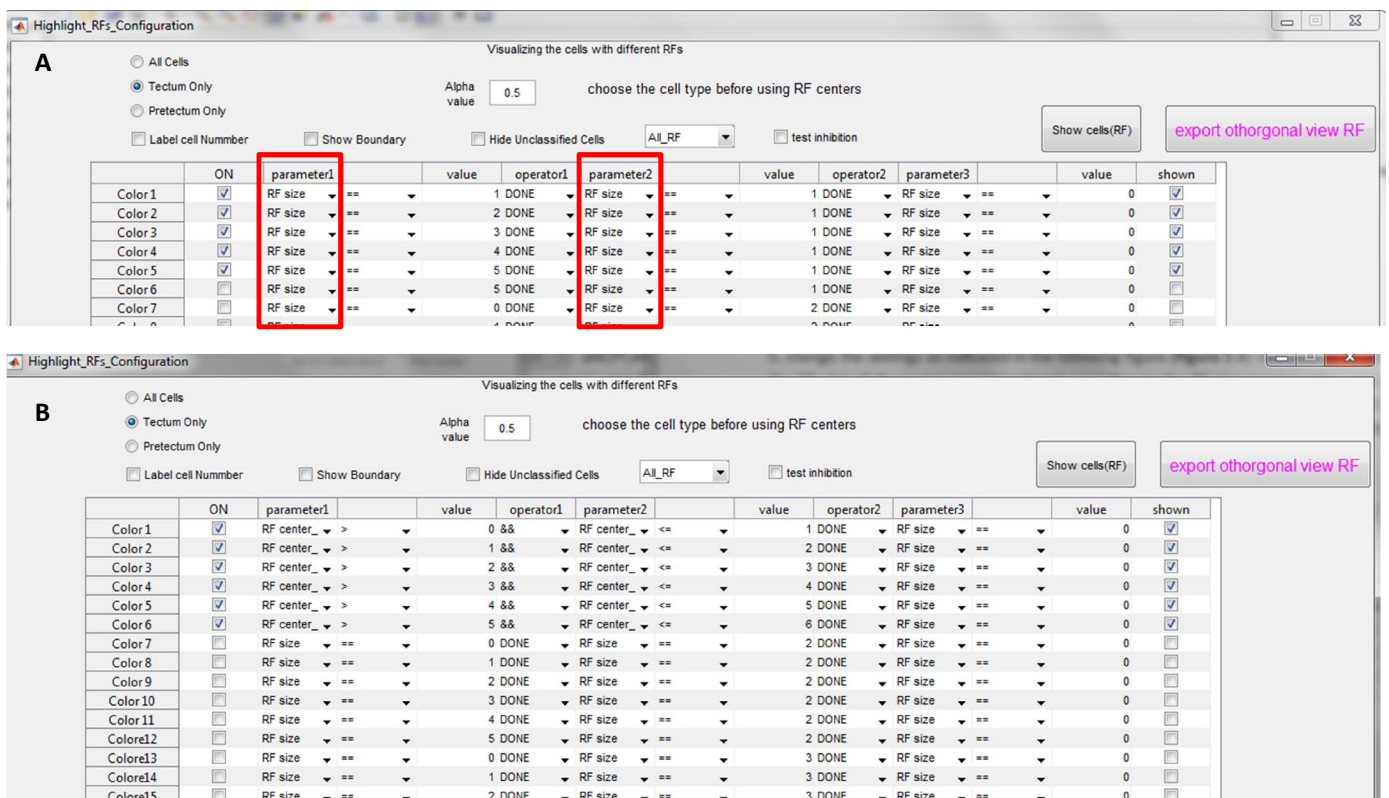


Figure 5. (A). Parameter settings for the user to show tectal neurons according to their RF size. **(B).** Parameter settings for the user to show tectal neurons according to their RF centers.

MOR behavioral data analysis

- 1) Add the OMR video data and the Matlab code into the Matlab pathway.
- 2) Run the Matlab code.
- 3) The 'crop out ROI' was activated. In these looping steps, the user needs to mask the dark fish head and dark areas which do NOT belong to the fish body (**Figure 6**).
 - a. Please draw an ROI (rectangular, shown in dim cyan) to mask the fish head.
 - b. Prompt 'was activated'; please type in number '1' if you need to mask any area (please avoid the areas which can be reached by the moving tail; this loop repeats until the user types in '0'). Otherwise, please type in '0' and wait for the results.

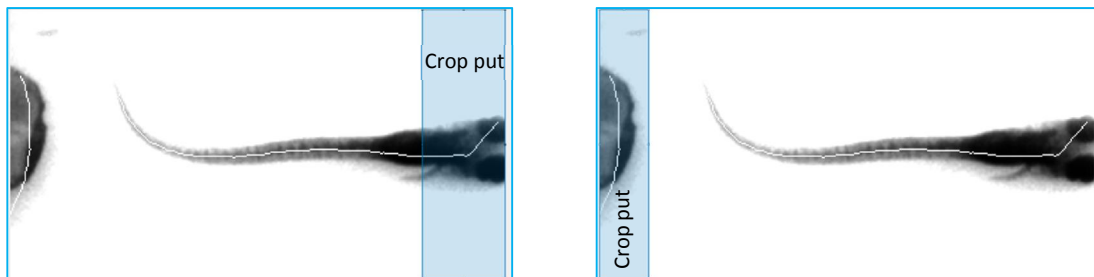


Figure 6. Areas masked by the users during the data analysis.